Neuropsychological dysfunction in children with sickle cell disease: The AANPAK project

Hijmans, C.T.; Oirschot, M. M. M.; Heijboer, H.; Peters, M.; Hendriks, C.J.; Last, B.F.; Oosterlaan, J.; Grootenhuis, M.A.; Fijnvandraat, K.

published in
Pediatric Clinics Amsterdam
2008

document version
Publisher's PDF, also known as Version of record

emma kinderziekenhuis AMC

Volume 19, number 1, 2008

PEDIATRIC CLINICS AMSTERDAM

Pediatric Clinics Amsterdam
is an edition from
Emma Kinderziekenhuis
AMC
Visit adress: Meibergdreef 9
1105 AZ Amsterdam
Post adress: Postbus 22660
1100 DD Amsterdam

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Published by
DCHG Partner in medische
communicatie
Zijlweg 70
2013 DK Haarlem
telefoon (023) 551 48 88
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e-mail: info@dchg.nl
internet: www.dchg.nl

Published three-monthly
ISSN 0928-7868
www.amc.nl

Editorial

In this issue of Pediatric Clinics Amsterdam we proudly present the papers of the young investigators who were selected during the Emma's Children Hospital scientific symposium 2008 to present their research in a master class. Although we are already looking forward to the Emma's Children Hospital scientific symposium 2009, we can also look back on a very successful symposium in 2008. Because of the large number of manuscripts available for publication in this issue, the paper written by the winner of the poster prize will be presented in the last issue of 2008, to be published in December.

Annet M. Bosch, editor in chief

Activation of the granzyme pathway in children with severe respiratory syncytial virus infection

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See full article: *Pediatr Res.* 63(6):650-5, June 2008, **and commentary:** Verbsky JW and Grossman WJ. RSV infection--an immune balancing act: Commentary on the article by Bem et al. on page 650. *Pediatr Res.* 2008 Jun;63(6):599-601.

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Introduction

Respiratory syncytial virus (RSV) is a major respiratory pathogen in infants and young children.[1] Although RSV infection is in general limited to the upper respiratory tract the disease may progress to the lower airways leading to acute hypoxemic respiratory failure. Despite decades of research the exact mechanisms that determine the development of severe RSV-lower respiratory illness (LRTI) in previously healthy children remain unclear.
One hypothesis proposes that activation of cell death pathways directed against RSV-infected cells and/or uninfected bystander cells contributes to disease severity.[2] Although regulated cell death or apoptosis seems an important mechanism for RSV clearance,

Welliver *et al.* identified marked expression of the apoptosis marker caspase-3 in airway epithelium of children with fatal RSV-LRTI, suggesting an imbalance in this process.[3,4]

Granzymes (Gr), serine proteases present in granules of effector lymphocytes, are involved in several host immune responses, including the activation of cell death (apoptotic) and inflammatory pathways.[5] Detection of free extracellular GrA and GrB is considered to reflect cytotoxic activation of the cell-mediated immune response.[6,7]

In the present study we hypothesized that severe RSV-LRTI in children is associated with local activation of the granzyme pathway by the cell-mediated host immune response. To test this, we investigated extracellular GrA and GrB and cellular expression of GrB in the respiratory tract of infants with RSV-LRTI.

Methods

Briefly, tracheal aspirate (TA) samples were obtained from 23 children with RSV-LRTI and 12 age-matched controls without a pulmonary condition. All patients were admitted to the intensive care unit for mechanical ventilation (MV) between November 2003 and March 2006. Infection with RSV was proved by direct immunofluorescence assay (Imagen, DakoCytomation, UK) of nasopharyngeal aspirate. Extracellular (activity of) GrA and GrB, interleukin (IL) -8 and extracellular caspase-cleaved cytokeratin-18 (CK18), a marker of epithelial cell apoptosis, were measured using (sandwich) immunoassays.

FACS analysis was performed in bronchoalveolar lavage fluid (BALF) samples from mechanically ventilated children with RSV-LRTI in the winters of 2006-2008. BALF was obtained by three subsequent instillations of 1 ml/kg of 0.9% saline through a wedged suction catheter passed through the endotracheal tube. BALF cells were stained with APC-labeled anti-CD3, PerCPCy5-labeled CD8 or CD4 and FITC-labeled anti CD16 and -56. For intracellular GrB staining cells were fixed and permeabilized with fixation/permeabilization solution and stained with PE-labeled anti-GrB mAb or a PE-labeled isotype control.

Results

Elevated GrA and GrB in TA during severe RSV-LRTI

The median (range) level of GrA in TA on the day of start of mechanical ventilation was 0.6 (0.3-14.3) ng/ml and 11.1 (0.3-98.4) ng/ml in controls and RSV-patients, respectively ($p<0.01$, Figure 1A). Likewise, the median (range) level of GrB in TA was higher in the RSV patients (69.0, 3.1-728.0 ng/ml) as compared to the controls (1.7, 0.5-39.6 ng/ml) ($p < 0.01$, Figure 1B). There was a significant correlation between the levels of GrA and GrB in TA (Spearman $r = 0.67$, $p<0.001$). In the RSV patients, the plasma levels of GrA (median 63.0, range 3.0-180.0 pg/ml) and GrB (median 35.0, range 13.0-92.0 pg/ml) on the first day of MV were significantly lower than the levels in TA ($p<0.001$ for both comparisons, data not shown).

To ascertain biological activity of GrA and GrB in the airways of RSV patients we measured active GrA and GrB with enzyme capture assays. There was a significant correlation between the levels of active GrA and total GrA antigen (Spearman $r= 0.82$, $p < 0.01$) (Figure 2A). In addition, we found a positive correlation between GrB activity and total GrB antigen (Spearman $r= 0.62$, $p = 0.05$) (Figure 2B).

There was a positive correlation between GrA and GrB and total WBC counts and IL-8 in TA in the RSV patients and this correlation tended to be stronger after the first days of MV (data not shown).

To study lung epithelial apoptosis in relation to extracellular granzymes, we analyzed the correlation between GrB and cleaved-CK18 in TA. Cytokeratin-18 is an epithelium-specific intermediate filament protein which is cleaved during apoptosis and may be subsequently released from cells into the extracellular space (8). There was no significant correlation between GrB and cleaved-CK18 in TA at any time (data not shown).

GrB expression by lymphocytes in BALF

T-cells and NK cells were detected in low numbers throughout the course of RSV-LRTI. GrB-positive cells were found predominantly among cells within the side/forward scatter region of the lymphocyte population by FACS analysis. In addition to $CD8^+$ T-cells and NK cells, $CD4^+$ T-cells were found to express GrB (data not shown).

Discussion

The main goal of this study was to determine whether severe RSV infection in children is associated with local activation of the granzyme pathway by the cell-mediated host immune response. We report high levels of proteolytic active extracellular GrA en GrB and marked expression of GrB by T-lymphocytes and NK cells in the respiratory tract of children during the course of severe RSV-LRTI. The levels of extracellular GrA and GrB correlated with total WBC counts and IL-8 levels in the airways after the acute-onset of RSV disease, but no association with the epithelium apoptosis marker cleaved-CK18 could be demonstrated.

The present study adds to our understanding of cell-mediated cytotoxic responses during severe RSV-LRTI in children. Numerous rodent studies have shown that both NK cells and CTLs are recruited to the lungs during primary RSV infection.[9-11] Graham *et al.* reported that although CTLs are involved in the clearance of RSV, depletion of these lymphocytes diminishes clinical illness upon exposure to RSV.[9] Treatment of RSV-infected CTL-depleted mice with high dose RSV-specific CTLs results in viral clearance, but augments lung injury by causing severe haemorrhage and neutrophilic infiltration.[12] These findings suggest that cell-mediated cytotoxic responses play an important role in RSV disease pathogenesis in mice, but the relevance in humans is unclear. Studies in vitro show that CTLs isolated from peripheral blood of RSV-infected infants lyse infected autologous target cells.[13,14] However, Welliver *et al.* recently reported the near absence of CTLs and NK cells in lung tissues from nine infants who died of RSV-LRTI.[4] Previous analysis of BALF samples of RSV-infected children showed that a small percentage of the total cells in the lungs is CD8-positive.[15] Similarly, in the present study we found low numbers of T-lymphocytes and NK cells in the lungs. However, despite these low cell counts, we

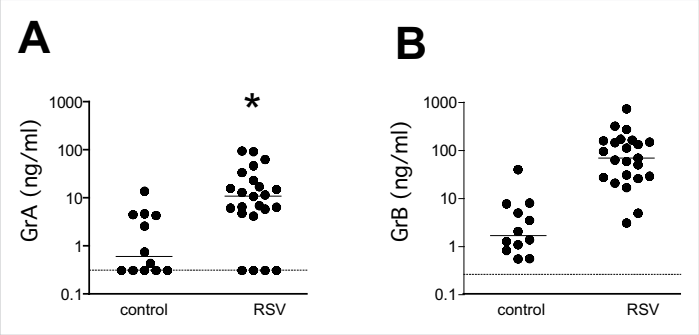

*Figure 1. A-B, levels of GrA and GrB (ng/ml) in TA from children without pulmonary condition (controls, n=12) or with RSV-LRTI (n=23) on the day of start of mechanical ventilation (MV). Bars represent median values. Dotted lines represent lowest detectable levels. * $p < 0.01$.*

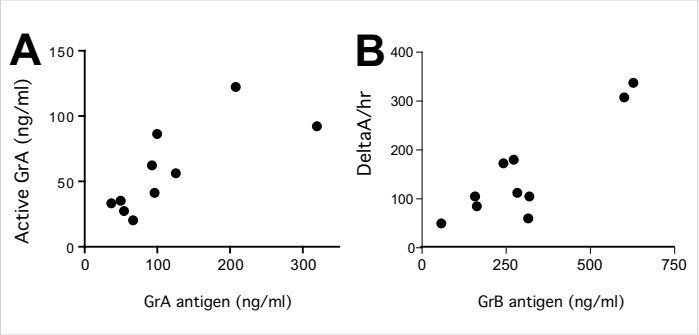

Figure 2. A, correlation between the levels (ng/ml) of GrA antigen and active GrA in TA (Spearman r = 0.82, $p < 0.01$). B, correlation between the level of total GrB antigen (ng/ml) and GrB proteolytic activity (deltaA per hr) in TA (Spearman r = 0.62, $p = 0.05$).

report high levels of extracellular granzymes and marked GrB expression in these cell populations in the respiratory tract.

The activation of the cell-mediated granzyme response in children with severe RSV-LRTI raises the possibility that this cell death pathway is involved in RSV disease pathogenesis. Direct killing of infected cells by granzymes is considered a major host defence mechanism against viruses in general. In mice, both GrA and GrB are essential in controlling ectromelia and cytomegalovirus infection.[16,17] Interestingly, viruses have been reported to encode proteins that inhibit granzymes, suggesting pathogen immune evasive adaptations.[18,19] On the other hand, enhanced death of infected cells and/or bystander cells may contribute to the development of disease by causing tissue dysfunction as has been suggested for severe inflammatory lung diseases.[20-22] Welliver *et al.* found marked expression of caspase-3 in airway epithelium of children with fatal RSV-LRTI, a finding consistent with studies performed in adult patients with ALI/ARDS.[4,23] The study of Welliver *et al.* suggests an imbalance in apoptosis during severe RSV-LRTI, but the involved cell death pathways remain unclear. Our findings may point toward a role for the granzyme pathway in apoptotic cell death during severe RSV-LRTI, but contrary to our expectations, we found no correlation between the levels of extracellular GrB and caspase-cleaved CK18 in the airways. The release of caspase cleaved CK18 is a surrogate marker of epithelial apoptosis but has not been studied extensively *in vivo*. The detection of cleaved CK18 in serum was found to correlate well with tissue damage in patients with hantavirus infection, but it is unclear how this protein behaves and reflects epithelial injury in other body fluids, including BALF.[24] Histological analysis of apoptosis to confirm our data is highly preferred but could not be performed in our study for obvious ethical reasons.

In addition to CTLs and NK cells, $CD4^+$ T-cells and basophils may express granzymes.[25,26] Recently, neutrophils were also shown to express granzymes[27], although this has been debated by others.[28] In the present study, GrB-positive cells were only found among lymphocytes. Our finding of GrB expression by $CD4^+$ T-lymphocytes is interesting because it suggests that the granzyme pathway may be utilized to modulate immune responses to RSV. Devadas *et al.* have shown that T_h2 cells express GrB which induces their own cell death.[29] Furthermore, they reported that GrB deficient mice have augmented T_h2 cytokine responses and lung cellular infiltrations in a model of allergic inflammation. Grossman *et al.* have shown that the granzyme pathway is exploited by natural T-regulatory cells ($CD4^+CD25^+$) against autologous activated $CD8^+$ and $CD4^+$ T-cells.[30] These findings suggest that granzymes function to control immune responses, in addition to their role in direct virus-infected cell killing.

In conclusion, severe RSV-LRTI in children is associated with high levels of proteolytic active extracellular GrA and GrB and expression of GrB by lymphocytes in the respiratory tract. These findings suggest that the granzyme pathway is activated by the local cell-mediated host immune response to RSV. Further studies should elucidate the exact role of granzymes in the lungs during RSV disease.

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Behavioral therapy for childhood constipation: a randomized controlled trial

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Results of this study were previously published in Pediatrics. 2008 May;121(5):e1334-41.

Introduction

Constipation is a symptom rather than a disease and often constitutes a major problem for the child and his family. Up to 84% of functionally constipated children suffer from fecal incontinence[1] and over one third exhibit behavior problems.[2,3]

Fearful reactions to defecation and stool withholding behavior are common in children with constipation.[4-9] Retained stools become progressively more difficult and painful to evacuate, leading to fear and avoidance of defecation.[10,11] This vicious cycle can be described as acquired behavior.

The objective of the present study is to evaluate behavioral therapy with laxatives compared to conventional treatment. It was hypothesized that behavioral therapy with laxatives would result in more success regarding constipation, stool-withholding behavior, and behavior problems.

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Methods

Patients

Between November 2002 and August 2004, children with functional constipation aged 4-18 years referred to the gastrointestinal outpatient clinic at the Emma Children's Hospital were eligible for enrolment. At entry, patients had to fulfill at least two of four criteria: defecation frequency <3 times per week, fecal incontinence ≥2 times per week, passage of large amounts of stool at least once every 7–30 days (large enough to clog the toilet) or a palpable abdominal or rectal fecal mass.[12]

Baseline assessment

Parents recorded frequency of stools and episodes of fecal incontinence in a bowel diary.

Intervention

A computer-based system was used to generate a sequence of random group assignment for consecutive patients. Conventional treatment (CT) and behavioral therapy (BT) consisted of 12 visits during 22 weeks. Both interventions employed similar laxative therapy.

Conventional Treatment

Children were treated with laxatives by the pediatric gastroenterologist and received education which included explanation that symptoms are not harmful and are common in children with functional constipation.[13]

Protocolized Behavioral Therapy

Pediatric psychologists of the psychosocial department of our hospital [14]

developed BT. The basic assumption is that phobic reactions related to defecation can be reduced and that adequate toileting behavior and appropriate defecation straining can be (re)acquired by teaching parents behavioral procedures and by behavioral play therapy with the child.

Clinical Outcomes

Primary outcome measures: 1) defecation frequency per week, 2) fecal incontinence frequency per week and, 3) successful treatment. Treatment was considered successful if patients achieved a defecation frequency of ≥3 times per week and a fecal incontinence frequency of
≤1 times per two weeks irrespective of laxative use. Secondary outcome measures: 1) stool-withholding behavior and, 2) behavior problems measured by the Child Behavior Checklist (CBCL/4–18).[15] The CBCL yields scores for a total problem scale and the 'broad band' syndrome scales internalizing and externalizing problems. The internalizing scale consists of items covering withdrawn behavior, somatic complaints, anxiety and depression. The externalizing scale consists of items covering delinquent and aggressive behavior. A T-score higher than 63 (90th percentile) indicates that a child needs professional help for his behavior problems. [15]

A post-treatment assessment took place in each intervention arm during the last visit and six months after completion of the 22 week-treatment (follow-up).

Statistical analysis

Intent-to-treat analyses were carried out. To determine the effect of treatment on primary and secondary outcome measures regression models were fitted with the factors treatment (conventional treatment, behavioral therapy), time (post-treatment, follow-up), and treatment-by-time. A p-value <0.05 was considered statistically significant.

Results

Sample

A total of 134 patients were assigned to conventional treatment or behavioral therapy (Figure 1). During treatment 2/64 (3.1%) in the CT group and 9/65 (13.8%) in the BT group discontinued intervention (p=.054). Baseline characteristics are presented in Table 1.

Primary outcomes

Baseline data are presented in Table 1. Defecation frequency increased from an average of 2.0 stools per week to 7.2 in the CT group and 5.4 in the BT group at post-treatment. Compared to the BT group, defecation frequency in CT was significantly higher (IRR=0.75, 95% CI=0.59-0.96; p=.021). Planned comparisons showed that this effect was mainly caused by a difference between interventions at post-treatment (7.2 vs. 5.4; p=.021), and not at follow-up (6.6 vs. 5.3; p=.150).

Fecal incontinence frequency dropped from an average of 15 per week at start of the study to 2.1 and 5.0 per week at post-treatment for CT and BT, respectively. From post-treatment to follow-up, fecal incontinence frequency increased to an average of 6.4 in CT and 8.6 in BT. No statistically significant difference was found between treatment conditions (p=.135).

At post-treatment, success rate was higher in CT (62.3%) than in BT (51.5%). No statistically significant difference between treatments was found, however (p=.249). At follow-up, the number of children successfully treated declined to 57.3% in CT and 42.3% in BT. Again, the difference proved statistically non-significant (p=.095).

Secondary outcomes

Baseline data are presented in Table 1. Stool-withholding behavior was reduced from baseline to follow-up in both treatment conditions; from over two-third of the children withholding their stools to 13.8% in CT and 10.6% in BT at post-treatment. The proportion of children with stool-withholding behavior did not differ between interventions (p=.654).

Over one-third of the children exhibited behavior problems (CBCL T-score>63) at baseline. At end of treatment, this percentage was decreased to 22.8% in CT and 21.9% in BT. At follow-up, BT was found to have influenced behavior problems significantly by reducing the proportion of children with these problems to 11.7% compared to 29.2% in CT (RR=0.42, 95% CI=0.18-0.96; p=.039).

The proportion of children with internalizing problems also declined from an average of 35.8% to 17.3% and 18.9% for CT and BT, respectively. At follow-up, this proportion increased in CT, but decreased further in BT (23.4% vs. 14.0%). However, no statistically significant effect was found for the effect of treatment condition (p=.600), nor for the influence of behavioral therapy at follow-up (p=.156).

The proportion of children exhibiting externalizing problems changed from an average proportion of 26.9% to 15.9% in CT and 15.6% in BT at post-treatment. Both treatments appeared equally effective in reducing externalizing problems (p=.990).

Conclusion

This study is the first large randomized controlled trial evaluating the clinical effectiveness of behavioral therapy with laxatives for functional constipation in childhood. The results indicate that this behavioral therapy with laxatives has no advantage over conventional treatment in treating childhood constipation. However, this study shows that behavioral therapy is superior in addressing behavior problems in constipated children.

Conventional treatment should remain the treatment of choice. Behavioral therapy may be considered when children concurrently experience behavior problems. Quality of care for chronically constipated children may be improved by adding a behavioral screening to the clinical evaluation of constipated children.[11,16] Positive screening should lead to consideration of behavioral therapy or referral to mental health services.

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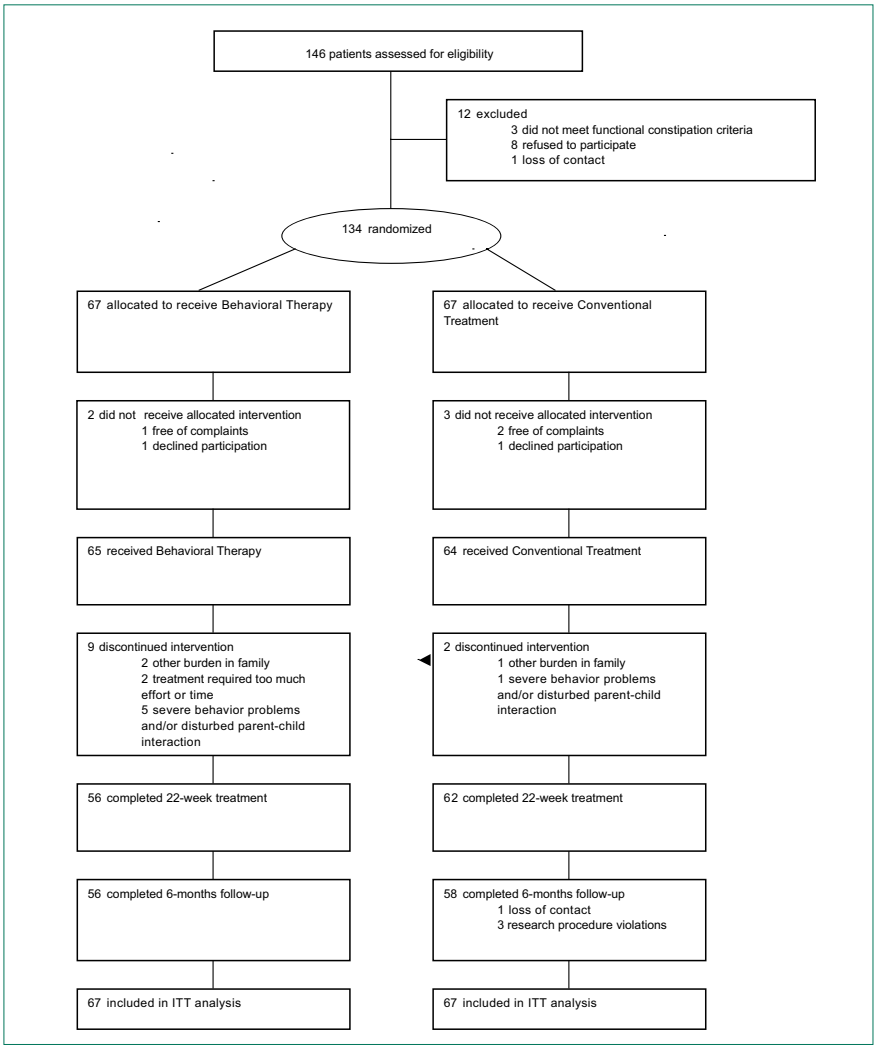

Figure 1

		Conventional Treatment (n=67)		Behavioral Therapy (n=67)		N	p-value
Demographics							
	Age, mean (SD), y	6.5	(2.1)	6.9	(2.5)	134	.367
	Boys, No (%)	37	(55.2)	39	(58.2)	134	.727
History							
	Age of onset constipation, mean (SD), y	3.0	(2.0)	2.8	(1.9)	134	.551
	Period of treatment, mean (SD), mo	17.1	(19.4)	18.7	(21.7)	129†	.673
	Positive family history, No (%)	28	(43.8)	33	(50.8)	131†	.338
Outcome							
	Defecation frequency/week, mean (SD)	1.9	(2.7)	2.0	(2.3)	134	.961
	Fecal incontinence/week, mean (SD)	15.6	(15.9)	15.0	(14.2)	134	.831
	Stool-withholding behavior, N (%)	44	(68.8)	43	(67.2)	128†	.850
	CBCL Total score, N (%)*	26	(38.8)	23	(34.3)	133‡	.591
	CBCL Internalizing score, N (%)*	25	(37.3)	23	(34.3)	133‡	.719
	CBCL Externalizing score, N (%)*	18	(26.9)	18	(26.9)	133‡	1.00
Additional clinical symptomatology							
	Painful defecation, No (%)	39	(65.0)	28	(43.1)	125†	.014
	Hard stools, No (%)	19	(32.2)	14	(22.2)	122†	.215
	Large amount of stool, No (%)	46	(68.7)	45	(67.2)	134	.853
	Abdominal pain, No (%)	46	(69.7)	46	(68.7)	133†	.897
	Day time urinary incontinence, No (%)	12	(17.9)	10	(14.9)	134	.641
	Night time urinary incontinence, No (%)	23	(34.3)	19	(28.4)	134	.456
Physical examination							
	Abdominal scybalus, No (%)	20	(31.3)	22	(35.5)	126§	.614
	Rectal scybalus, No (%)	27	(49.1)	38	(58.5)	120§	.305

Table 1. Baseline characteristics of children allocated to conventional treatment or behavioral therapy.
Abbreviations. SD: Standard deviation; y: year; mo: month; CBCL: Child Behavior Checklist.
† *Missing characteristics were unknown to parents.*
‡ *One CBCL questionnaire was not filled out.*
§ *Missing physical examination, because the child was too anxious to perform examination.*
* *Proportion children with CBCL T-score >63 (90th percentile)*

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Hidden consequences of success in pediatrics: parental Health Related Quality of Life. Results of the CARE project

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Introduction

In The Netherlands at least 500,000 (14%) of all children grow up with a chronic disease.[1] Recent data from the United States show that at least 7% (5 million children) of all children have a limitation of activity due to a health condition, the number of chronically ill children being even higher.[2] The number of children with a chronic disease is likely to increase further due to medical improvement, genetic, social and behavioral changes.[2] For parents, learning that their child has a chronic and potentially life threatening disease is a very stressful and potentially traumatic event.[3] Besides emotional impact, having a chronically ill child also influences family and social life, as parents provide most of the daily care for these children.[4,5] The care-giving role combined with family life, an occupational career and social life can be very stressful for parents.[6,7] Parents report a lower quality of life and experience physical and emotional strain.[8,9] Parental physical, emotional and social health also influences the health and well-being of their children.[3,10] This results in a contradictory situation as for parents it is becoming more difficult to fulfill all tasks, while on the other hand their role in providing care is of increasing importance for the health and well-being of their children.

Analysis of mean scores showed a lower Health Related Quality of Life (HRQOL) for parents of chronically ill children. In this paper we asses the prevalence of parents with an impaired quality of life based on the 25th percentile of the norm population. This information will provide insight into the degree of parental HRQOL impairment within different groups.

Methods

Participants

Parents of chronically ill children were recruited between January 2006 and September 2007 in the Emma Children's Hospital in Amsterdam, The Netherlands, and through patient organizations. Chronic illness was defined according to Mokkink et al.[1,11], including the following criteria: the disease occurs in children aged 0-18 years, the diagnosis is based on medical scientific knowledge, is not (yet) curable and has existed for at least three months, or will probably continue longer, or at least three disease episodes have occurred the last year. We selected ten different chronic diseases in childhood: asthma, diabetes, Down syndrome, Duchenne's muscular dystrophy, end stage renal disease, metabolic diseases, profound multiple handicaps, sickle cell disease, spina bifida, and survivors of a brain tumor. Inclusion criteria were: (1) the chronically ill children were aged between 1-19 years old, (2) were diagnosed >1 year before inclusion in the study, (3) the children lived at home (4) parents were able to fill out the questionnaire in Dutch or English.

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Parents in the comparison group were eligible if their child (1) was not chronically ill, (2) was between 1-19 years old (3) was living at home (4) parents were able to fill out the questionnaire in Dutch or English.

Procedure

Parents received an introductory letter explaining the aim of the study and requesting their participation. The letter was accompanied by the questionnaire, an informed consent form and a stamped self-addressed envelope. The comparison group consisted of parents of healthy children from two elementary schools and one high school located within 50 kilometers of our hospital. The schoolchildren took an envelope home for their parents, including the introductory letter, the questionnaire, the informed consent form and the stamped self-addressed envelope.

Measurement

HRQOL was assessed with the 'TNO-AZL Questionnaire for Adult's Health related Quality of Life' (TAAQOL).[12] The questionnaire measures health status problems weighted by the impact of problems on well-being on 12 multi-item scales of which we present six in this paper: sleep, social functioning, daily activities, vitality, positive emotions and depressive emotions.

Each item consists of two parts: the first part assesses the prevalence of a health problem or limitation in the past month, the second part the emotional response to the health problem or limitation. Answers were scored on 4 point scales. The scales vitality, positive emotions, depressive emotions and aggressiveness only assess the occurrence of the feelings in the past month. Higher scores indicate a better HRQOL. The Cronbach's alphas in the present study were mainly satisfactory to good, ranging from 0.60-0.96. The psychometric properties, validity and reliability, of the TAAQOL were satisfactory.[12]

Statistical analysis

First, scales were constructed and missing data were imputed based on the guidelines of the TAAQOL. During calculation of the scale scores one missing combined-item score was allowed for. The missing score is replaced by the mean value of the non-missing item scores. Demographic data of the study and comparison group were compared using Chi-square tests for categorical data and t-tests for continuous data. We created a distinction between parents 'at risk' and those 'not at risk' for an impaired HRQOL, based on percentile norms of the healthy population.[13] A parent in the comparison group who scores below the value of the 25th percentile is placed in the quarter of the most impaired population. We compared the percentage of parents in the disease samples scoring below the value of the 25th percentile of the norm population, using binomial tests $p<0.008$ (0.05/6). We used the Statistical Package for Social Sciences (SPSS) version 14.0.

Results

Participants

The number of participants is shown in Table 1. The average response rate was 54%. Overall, the groups had similar demographics ($p<0.1$) except for educational level, with a larger proportion of highly educated parents in the comparison group (Table 1). Gender distribution in Duchenne, sickle cell disease and survivors of a brain tumor statistically differed from the comparison group. Furthermore, parents of children with sickle cell disease and end stage renal disease were more often single and had a lower educational level than the comparison group. Parents of children with metabolic disease also had a lower educational level. Parents of children with Down syndrome had more children than the comparison group. Furthermore, the age of parents of children with asthma, sickle cell disease and metabolic diseases was significantly lower than in the comparison group.

Proportion of parents of chronically ill children at risk for HRQOL impairment

Table 2 shows the percentages of parents at risk for HRQOL impairment for the scales sleep, social functioning, daily activities, vitality, and positive and depressive emotions. The total group of parents of chronically ill children had significantly higher percentages than the comparison group, ranging from 35% to 54%. In the disease groups, parents of children with asthma, metabolic disease, and sickle cell disease had significantly higher percentages than the comparison group on most scales, these parents were therefore considered at risk for an impaired HRQOL. Highest percentages of parents with low HRQOL scores were found on the scales social functioning and depressive emotions.

Discussion

The aim of the present study was to determine the number of parents with an impaired HRQOL. The results show that parents of chronically ill children have a seriously low HRQOL almost twice as often as parents of healthy children, and are therefore at risk for HRQOL impairment. Subgroup analysis shows similar results. Although not all differences are statistically significant, they indicate a trend towards low HRQOL scores for parents of chronically ill children. The definition of parents at risk for an impaired HRQOL was based on the value of the 25th percentile of all scales of the comparison group. There is no gold standard for a good or bad HRQOL, however, this definition is considered to be an appropriate way to differentiate between individuals with higher scale scores from individuals with lower scale scores.[13] Our results are in line with other studies describing more problems with depression, social and emotional functioning in parents of chronically ill children.[6,8,9] It should be noted that besides the above mentioned difficulties, there are also parents who seem to cope well with their child's disease. Both positive and negative determinants of HRQOL need to be estimated in future research.

A salient result is that parents of children with metabolic disease report a low HRQOL on almost all subscales. This might be explained by the hereditary and progressive nature of these diseases (lysosomal storage diseases, organic acidurias, mitochondrial respiratory chain defects), with an uncertain health status for several children, which leads to growing strain and increasing caregiver burden over time.[14] These care giving demands are extensive and are known to influence parental psychological and physical health.[4]

Parents of children with sickle cell disease are also at risk for HRQOL impairment. This could also be explained by their demographics, which differ most from the comparison group. In addition, parents of children with sickle cell disease in the Netherlands are often

	Comparison group	Parents chronically ill children	Asthma	Brain tumors	Diabetes	Duchenne muscular dystrophy	Down syndrome	End stage renal disease	Metabolic disease	Profound complex handicaps	Sickle cell disease	Spina bifida
	n=435	n=544	n=90	n=38	n=24	n=57	n=101	n=21	n=118	n=13	n=61	n=21
Gender (Female)	362 (83%)	453 (83%)	78 (87%)	34 (90%)	21 (88%)	42 (74%)	87 (86%)	20 (95%)	90 (76%)	9 (69%)	53 (88%)	19 (91%)
Married/Partner	386 (87%)	470 (89%)	75 (83%)	32 (84%)	23 (96%)	56 (98%)^	99(98%)^	16 (76%)	110 (93%)	12 (92%)	30 (50%)^	17 (81%)
Age Years (SD)	43.7 (5.5)	42.0 (6.4)*	42.2 (6.6)	45.8 (5.5)*	45.0 (6.6)	44.0 (5.6)	41.6 (4.9)*	42.8 (3.6)	41.3 (7.0)*	43.4 (5.2)	38.2 (7.3)*	41.0 (6.4)*
Educational level												
Lower	86 (20%)	140 (26%)^	15 (17%)	9 (24%)	7 (29%)	15 (26%)	20 (20%)	7 (37%)^	29 (25%)	3 (25%)	30 (50%)^	5 (24%)
Intermediate	165 (39%)	220 (41%)	40 (45%)	17 (45%)	8 (33%)	20 (35%)	40 (40%)	10 (52%)	50 (43%)	3 (25%)	22 (37%)	10 (48%)
Higher	178 (41%)	176 (33%)	33 (38%)	12 (31%)	9 (38%)	22 (39%)	40 (40%)	2 (11%)	38 (32%)	6 (50%)	8 (13%)	6 (28%)
Children per family												
Mean (SD)	2.2 (0.8)	2.2 (0.9)	2.2 (0.9)	2.0 (1.0)	2.1 (0.7)	2.2 (1.0)	2.5 (0.9)*	2.1 (0.7)	2.2 (0.9)	2.4 (1.0)	2.3 (1.2)	2.5 (0.8)
Age chronically ill children												
Yr (SD)	-	10.0 (4.5)	10.7 (4.3)	14.6 (4.4)	12.6 (4.4)	11.6 (4.0)	7.3 (1.4)	13.1 (4.1)	8.7 (4.4)	9.8 (4.2)	9.2 (4.5)	9.9 (4.8)
Time since diagnosis												
Yr (SD)	-	7.8 (4.3)	8.9 (5.0)	8.1 (3.8)	8.0 (5.5)	8.4 (4.3)	7.6 (1.3)	10.9 (5.3)	6.1 (4.0)	10.0 (3.8)	7.5 (4.4)	9.5 (5.1)

Table 1 Demographics

** p<0.1 for disease group in comparison with the reference group (t-test); ^ p<0.1 for disease group in comparison with reference group (chi square)*

migrant families, who generally are known to have a lower socioeconomic status (SES).To account for SES, we have previously compared this group of parents to a SES matched control group[15], which showed that parents of children with sickle cell disease have a lower HRQOL on the scales daily activities, vitality and depressive emotions.

Some limitations of this study should be addressed. First, although we describe ten different groups of parents of chronically ill children, we cannot easily generalize the results to all parents of chronically ill children as more than 280 ICD-10 diagnoses meet the criteria of chronic childhood disease.[1] Yet, the outcome of this study indicates an overall burden for parents across disease groups. Second, respondents in this study were mainly mothers, thus a more thorough exploration of the HRQOL of fathers is desirable. Third, parents in this study had a higher educational level than the average Dutch population (Lower: 33%, Intermediate 41%, Higher 25%) (16), with the exception of parents of children with end stage renal disease and sickle cell disease. The percentage of parents born in the Netherlands is also higher than average in the Dutch population. Our expectation is that participants in this study had a better SES than average in the Netherlands. A lower individual and neighborhood SES level is associated with worse health status.[17] Therefore in our study we probably show the HRQOL of parents with a relatively good SES and health status and presumably give an underestimation of the HRQOL impairment. A fourth limitation is the average response rate of 54% in the disease groups. We have little information on non responders, although the higher educational level in comparison to the Dutch average indicates a selection bias towards parents with higher educational levels. Finally, we must note that several sample sizes were small; these data do however generate the hypothesis of more parents being at risk for HRQOL impairment in these groups.

Clinical and future implications

In conclusion, parents in our study group report seriously impaired HRQOL. Parental mental functioning is known to influence their children's health[18] and adjustment[19], both for chronically ill and healthy children. Since parents of chronically ill children report higher stress levels than parents of healthy children, chronically ill children and their siblings are at higher risk for additional health and adjustment problems.

In our opinion pediatricians as well as GP's should be aware of the impact of caring for a chronically ill child on parental HRQOL and of the multitude

Condition	N	Sleep	Social functioning	Daily activities	Vitality	Positive emotions	Depressive emotions
Parents healthy children[1]	433	25%	25%	25%	25%	25%	25%
Chronically ill children	544	42%**	52%**	45%**	41%**	35%**	55%**
Asthma	90	45%**	37%	45%**	40**%	34%	43%**
Survivors of brain tumor	38	42%	40%	16%	19%	24%	49%**
Diabetes	24	42%	50%	42%	33%	29%	46%
Duchenne's muscular dystrophy	57	40%	58%**	40%	46%**	38%	59%**
Down syndrome	101	33%	53%**	49%**	34%	28%	37%
End stage renal disease	21	44%	61%**	44%	50%	33%	67%**
Metabolic diseases	118	44%**	58%**	56%**	46%**	41%**	69%**
Profound complex handicap	13	33%	55%	40%	50%	55%	40%
Sickle cell disease	61	54%**	55%**	39%	52%**	47%**	75%**
Spina Bifida	21	43%	57%	52%	48%	29%	52%

Table 2 Percentage of parents of chronically ill children at risk for HRQOL impairment

[1] 25th percentiles of parents of healthy children are not exactly 25%, due to distribution of scale scores. Percentiles approach 25th, ranging from 18th- 31st percentile.

***p<0.008 (0.05/6) for parents in disease group in comparison with parents of healthy children, binomial test*

of problems parents report. Parental functioning should be part of clinical practice in pediatrics. Moreover, attention should be paid to parental functioning as their resilience may be impaired due to sleeping problems and problems with vitality and depressive emotions. Family oriented programs which have been developed in e.g. psycho-oncology[20] should be introduced in other disease populations as well. Professionals working with parents of chronically ill children should pay attention to the difficulties parents experience and provide supportive care if necessary.

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Neuropsychological dysfunction in children with sickle cell disease: The AANPAK project

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Introduction

Sickle cell disease (SCD) is a hereditary red blood cell disorder that occurs predominantly in people of African ancestry.[1] The clinical picture of SCD is characterized by chronic hemolytic anemia and vascular occlusion, causing recurrent painful episodes (vaso-occlusive crises) and irreversible organ damage.

Therapeutic options for SCD comprise hematopoietic stem cell transplantation, scheduled blood transfusions and administration of hydroxyurea. Hematopoietic stem cell transplantation may have severe side effects and therefore most physicians are reluctant to its administration. Scheduled blood transfusion is another therapeutic option. Unfortunately, it is associated with iron overload, which leads to cardiac and endocrine dysfunction if adequate chelation is not installed. Finally, hydroxyurea is a therapeutic modality that has proved to reduce the rate of painful vaso-occlusive crises in bone.

The most devastating complication of SCD is cerebral infarction. At the age of 18 years, cerebral infarcts are present on MRI scans in one third of SCD patients, yet most of these infarcts are not accompanied by focal neurological deficits. These so-called silent infarcts appear to be associated with diminished neurocognitive functioning and an increased

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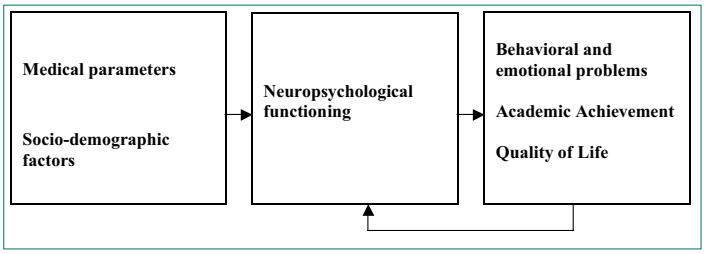

Figure 1. Theoretical model of factors influencing neuropsychological functioning in children with SCD.

Factors	Instruments
Medical parameters	Cerebral MRI, hematologic parameters
Socio-demographic factors	Sex, age, SES, maternal education, ethnic background
Neuropsychological functioning	Intelligence, attention, inhibition, visuospatial memory, verbal working memory/auditory processing, planning/organization, visual-motor integration
Behavioral and emotional problems	- Child Behavior Checklist (CBCL) - Teacher Report Form (TRF) - Vragenlijst voor Gedragsproblemen bij Kinderen (VvGK) [Questionnaire for Behavioral Problems in Children].
Academic achievement	CITO scores [Dutch Secondary School placement exam scores]
Quality of Life	Kidscreen

Table 1. Factors and corresponding instruments used in the AANPAK project

risk of new infarcts.[2,3] Neurocognitive deficits, such as memory problems or a limited attention span, may hamper the development and academic achievement of children with SCD. Early detection of neurocognitive deficits is important, providing an opportunity for early intervention with adequate support. This will have a positive effect on the development and academic achievement of these children. This is important, because academic underachievement may jeopardize full participation of children with SCD in society.

The unpredictable course of SCD places a heavy strain on affected children and their families. Besides the medical problems, most families with a child with SCD have to cope with financial and social problems, as the majority of these families belong to immigrant communities with a lower socio-economic status (SES) and 57% of these families are single parented.[4] The combined effects of neuropsychological dysfunction, stress caused by the disease and socio-demographic factors may lead to behavioral and emotional problems in children with SCD. If behavioral and emotional problems arise, it may be difficult for parents and professionals involved in the care of these patients to distinguish between the causes of these problems. However, it is important to differentiate between different causes because they may require different interventions.

In August 2007 the AANPAK project (AAndacht voor NeuroPsychologische functiestoornissen bij Kinderen met sikkelcelziekte) was initiated at the Comprehensive Sickle Cell Care Centre of Emma Children's Hospital, Academic Medical Center, in Amsterdam. The project aims at identification of neuropsychological consequences of SCD. Furthermore, behavioral and emotional problems, academic achievement and quality of life of SCD patients are evaluated. The ultimate goal of the project is to offer these patients specific care matching their needs.
The theoretical model of the interrelatedness of all these aspects is depicted by the three boxes in Figure 1. Within this model behavioral and emotional problems, academic achievement and quality of life are regarded as outcomes of neuropsychological functioning, which in itself is regarded as an outcome of medical and socio-demographic determinants. Factors depicted in the right box of Figure 1 may influence neuropsychological functioning (arrow below third box directed at middle box).
This theoretical model forms the base for three research questions of the AANPAK project:

1. What are the differences between children with SCD and healthy controls (matched for ethnicity and socio-economic status) in neuropsychological functioning, behavioral and emotional problems, academic achievement and quality of life?
2. What is the association between neuropsychological functioning on the one hand and behavioral and emotional problems, academic achievement and quality of life on the other hand in the total study group (containing both children with SCD and healthy controls)?
3. Which biological determinants (medical parameters) of neuropsychological functioning can be identified in children with SCD?

The data that will be acquired to answer these questions are given in Table 1. These data will be obtained from patients with SCD and from healthy controls.

As data collection is currently ongoing and full data cannot yet be analyzed, this manuscript presents the preliminary results of the frequency and severity of

	Internalizing		Externalizing	
	n	%	n	%
Sex				
Male (n=49)	7	14	10	20
Female (n=27)	5	19	5	19
Age in years				
6-11 (n=43)	5	12*	8	19
12-18 (n=33)	7	21	7	21
Disease severity				
Severe form of SCD (n=61)	12	20**	10	16
Moderate form of SCD (n=15)	0	0	5	33
Total group (n=76)	12	16	15	20

Table 2. Proportion of SCD children with problem scores within the clinical range on the TRF broad-band scales.
** Significant difference at p<0.01 between children aged 6-11 and 12-18.*
*** Significant difference at p<0.05 between children with severe and moderate form of SCD.*

behavioral and emotional problems in children with SCD, as reported by their teachers.

Methods

Patients

Patients aged 6-18 years with SCD (HbSS, HbS-βo-thalassemia, HbS-β+-thalassemia or HbSC) who were treated at the Comprehensive Sickle Cell Care Centre of Emma Children's Hospital, Academic Medical Center, in Amsterdam were eligible for inclusion. After parental informed consent was given, questionnaires were sent to their teachers from August 2007 onwards.

Instruments

The Teacher Report Form (TRF)[5] was used. This questionnaire provides scores on two broad-band scales: Internalizing (Anxious/Depressed scale, Withdrawn/Depressed scale and Somatic Complaints scale) and Externalizing (Rule-breaking Behavior scale and Aggressive Behavior scale). Adequate psychometric properties for this rating scale have been established.
T-scores higher than 63 (above the 90th percentile) represent problems within the clinical range and indicate that a child needs professional help. In the Dutch norm group 9% of the children had scores within the clinical range on both broad-band scales.

Statistics

Frequency distributions were calculated to determine the number of patients with problem scores within the clinical range. The chi-square test was used to determine differences according to sex, age and disease severity.

RESULTS

124 Children with SCD were eligible for inclusion. Up to January 2008, 76 questionnaires were returned (response rate 61%). The questionnaires concerned 49 boys and 27 girls (mean age 11.5 years, SD 3.5). Sixty-one (80%) children have a severe form of SCD (HbSS or HbS-beta°-thalassemia) and 15 (20%) children have a moderate form of SCD (compound heterozygous form: HbSC or HbS-beta⁺-thalassemia).

In Table 2 the proportion of children with clinical problem scores is given for the two broad-band scales. In the total group 16% of patients have internalizing problems and 20% of patients have externalizing problems. These proportions are significantly higher than those in the Dutch norm group. No differences between boys and girls were found.

In patients above 12 years a significantly higher proportion had internalizing problems (21%) as compared with in younger patients (12%). The proportion of patients with a severe form of SCD with internalizing problems (20%) was significantly higher than that in patients with a moderate form of SCD (0%).

Discussion

First preliminary results show an increased frequency of both internalizing and externalizing problems, especially in adolescents with SCD. However, these preliminary results are obtained only by teachers´ reports and do not take the opinion of the parents into account. When questionnaires that are filled in by the parents are available this may confirm or change the findings.
Moreover, since these preliminary results are derived exclusively from children with SCD, we cannot yet determine whether behavioral and emotional problems are a specific consequence of SCD or whether they are a result of the socio-demographic situation of these children. This will be analyzed when data from healthy controls are available.

Conclusions

These findings indicate that according to the teachers´ report almost 1 out of 5 children with SCD has behavioral and /or emotional problems within the clinical range and needs professional help. Children with a severe form of SCD and older children (12-18 years) seem to be at increased risk of development of internalizing problems.

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Effect of body position changes on triggering of postprandial gastroesophageal reflux and gastric emptying in the healthy premature neonate

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List of abbreviations

EGJ: esophagogastric junction
GE: gastric emptying
GER: gastroesophageal reflux
GERD: gastroesophageal reflux disease
LES: lower esophageal sphincter
LLP: left lateral position
PPH: postprandial hour
RLP: right lateral position
TLESR: transient lower esophageal sphincter relaxation

Introduction

Gastroesophageal reflux (GER) is present in all children. Typical GER symptoms, such as regurgitation, are normally mild and relatively harmless and therefore considered to be physiological.[1] However, in a subgroup of infants, GER can cause more severe symptoms, such as feeding problems and failure to thrive, as well as complications, such as esophagitis. It is then referred to as GER disease.

The acidity of GER episodes is thought to be a major pathophysiological factor in the development of these symptoms and complications. In infants, gastric contents are buffered by milk in the postprandial period.[2,3] Consequently, GER is likely to be weakly acidic (4 < pH < 7) in the first hour after a meal, while acid GER (pH<4) occurs more often in the late postprandial period.[4]

In all age groups, gastric distension induced (e.g. after a meal) transient lower esophageal sphincter relaxation (TLESR) is the most important underlying mechanism of GER.[5,7]

We previously studied the effect of left/right body positioning on GER and TLESR triggering in premature infants and found that right-lateral positioning (RLP) was associated with an increased proportion of liquid reflux compared to left lateral positioning (LLP).[8] This was probably due to the presence of gastric contents above the level of the esophagogastric junction in the latter position, as demonstrated radiologically by Ewer and colleagues.[9] However, a more interesting finding of our previous study, was that the overall triggering of TLESRs following feeding was significantly greater and gastric emptying (GE) of the feed significantly faster in the RLP.

The aim of this study was to investigate the effects of changing body position on the triggering of TLESRs, GER and GE, in order to identify a positioning regime that could both promote GE and decrease the number of acid GER episodes occurring during the late postprandial period, when GER becomes more acidic.

Methods

Subjects and baseline characteristics (Table 1)

We studied 10 preterm infants (7 male; 3 female) who did not experience any symptoms related to GER or other gastrointestinal diseases and were healthy apart from their prematurity. All subjects were studied at the Women's and Children's Hospital in Adelaide, Australia. The subjects had a median gestational age of 31.5 (range: 27-36) weeks. Postnatal age at commencement of the study was 23 (11-62) days resulting in a corrected age of 36 (33-38) weeks). The median weight at the time of the study was 2415 (2130 – 2800) g. For ethical reasons, all infants had to receive at least one of their daily feeds by gavage. The parents or guardians gave written informed consent before the commencement of each study and the protocol was approved by the Research Ethics Committee of the Women's and Children's Hospital.

Measurement Techniques

Esophageal impedance and manometry

A combined multichannel intraluminal impedance and manometry catheter (outer diameter: 2 mm) that also allowed for gavage feeding was developed for the purpose of this study (Figure 1). The assembly

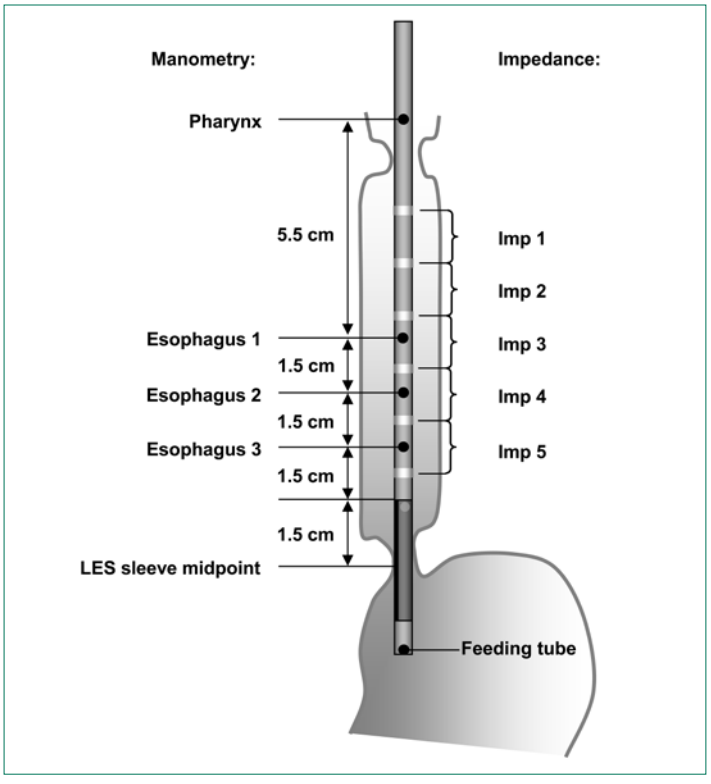

Figure 1: Catheter design. Imp: impedance segment; LES: lower esophageal sphincter

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No	Sex	PMA	PNA	BW	Weight	Feed	Volume	Frequency	1st protocol
1	Male	33	24	1950	2500	Formula	70	6	RLP first
2	Female	28	45	1340	2330	EBM	60	7	LLP first
3	Male	33	21	1730	2210	EBM	65	6	RLP first
4	Male	36	14	2450	2630	EBM	70	6	LLP first
5	Female	29	44	1205	2330	EBM	50	7	RLP first
6	Male	29	52	1370	2520	Formula	70	6	LLP first
7	Female	27	62	1050	2530	Formula	70	6	RLP first
8	Male	30	22	1920	2130	Formula	55	7	RLP first
9	Male	34	13	2830	2800	EBM	80	6	LLP first
10	Male	34	11	2370	2320	Formula	65	7	LLP first

Table 1: Demographics and randomization.
No: studynumber; PMA: post menstrual age (wks); PNA: post natal age (days); BW: birthweigt (g); Weight: weight at first studyday (g); EBM: expressed breast milk; Volume: volume of feed (ml); Frequency: feeds/day; 1st protocol: protocol followed during first study day. RLP: right lateral position; LLP: left lateral position.

consisted of a water-perfused manometric sleeved catheter. Electrode rings allowed for the recording of 5 segments of intraluminal impedance throughout the esophagus. A feeding lumen was incorporated with its opening at the distal end of the assembly.

Gastric emptying rate

Gastric emptying rate was determined with the ^{13}C-Na-octanoate breath test. This test has been described in detail elsewhere and has proved to be a reliable and reproducible non invasive means of testing gastric emptying.[10,11]

Experimental protocol

All infants were studied twice on two consecutive days and subjected to two positioning protocols in a randomized cross-over fashion (Figure 2). The assembly was passed transnasally into the stomach and positioned with the sleeve straddling the LES. The infants were then positioned in either the right lateral position (RLP) or left lateral position (LLP) and gavage fed their normal feed (expressed breast milk or formula). Feed volume was identical for both studies and the feed was infused by the same research nurse, who tried to keep the infusion velocity constant. One hour following the end of the feed, the infant's position was changed to the opposite lateral side and manometry and impedance recordings continued for a further two hours.

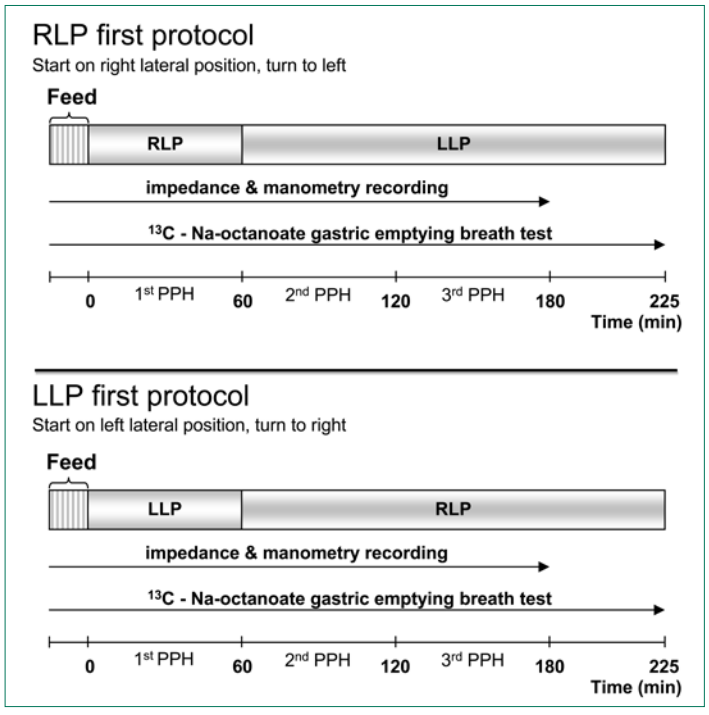

Figure 2: Experimental protocol. Note that all infants were studied according to both protocols in a cross over fashion. The feeding time was dependent on the volume of feed primarily. RLP: right lateral position; LLP: left lateral position; PPH: postprandial hour

Data analysis

All details that could identify the subject and all information regarding the protocol were removed from each tracing before analyses. Manometry and impedance tracings were analyzed for GER and TLESRs using established criteria.[12,13] Manometry and impedance data were compared between the RLP and the LLP. To this end, data from the 1st hour of the RLP first protocol were combined with the 2nd and 3rd hour of the LLP first protocol and these were compared with the data from the 1st hour of the LLP first protocol and 2nd and 3rd hour of the RLP first protocol. In addition, all data were compared between the two protocols and between the first and second postprandial hour (PPH) in both protocols.

Statistical analysis

Normally distributed data are presented as means ± SD and are compared between the protocols or between sides using the paired t-test. Non parametric data are presented as median (range) and are compared with the Wilcoxon's matched pairs signed ranks test. A p-value of less then 0.05 was considered statistically significant.

Results

Feeding volumes and time

Feed volumes given to the infants were identical on both study days, ranging from 50 - 80 ml between infants (median: 67.5 ml) Administration of the feed took 12.3 (7.5–36.2) minutes by hand-driven syringe. The time taken to administer feeds was similar in the two protocols (RLP first: 13.1 (7.5-36.2) min vs. LLP: 12.3 (8.0-25.8) min., p=0.770).

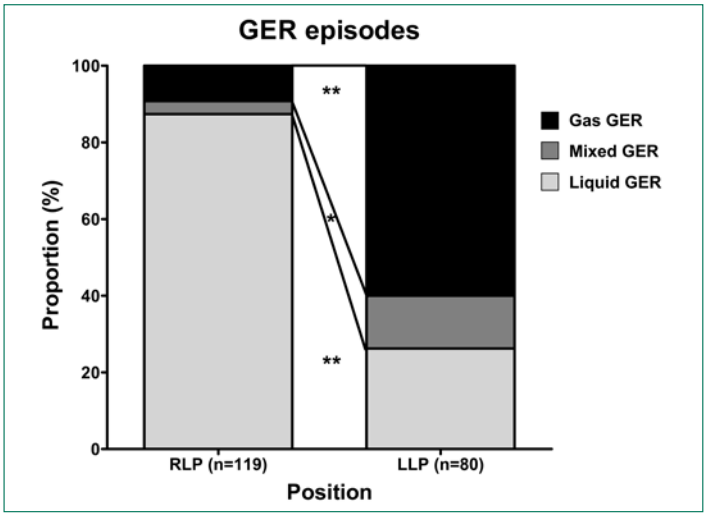

*Figure 3: Percentages of liquid, mixed and gas GER attributing to the total number of GER episodes on the LLP and the RLP. (Absolute number of GER episodes given in parentheses). GER: gastro-esophageal reflux; LLP: left lateral position; RLP right lateral position. * p<0.01; ** p<0.005, Wilcoxon's matched pairs signed rank sum test.*

		RLP first protocol	LLP first protocol	p value
Overall	**TLESR**	7.5 (4.0-21.0)	9.0 (2.0-15.0)	NS (0.193)
	All GER	9.0 (5.0-28.0)	7.0 (3.0-15.0)	NS (0.322)
	Liquid GER	6.0 (2.0-13.0)	7.0 (2.0-13.0)	NS (0.469)
	Mixed GER	0.0 (0.0-5.0)	0.5 (0.0-2.0)	NS (0.813)
	Gas GER	4.0 (1.0-10.0)	1.0 (0.0-6.0)	NS (0.055)
1st PPH		RLP:	LLP:	
	TLESR	4.0 (2.0-8.0)	2.5 (0.0-6.0)	0.031
	All GER	5.5 (2.0-15.0)	2.5 (1.0-5.0)	0.004
	Liquid GER	5.5 (2.0-13.0)	2.0 (0.0-4.0)	0.004
	Mixed GER	0.0 (0.0-2.0)	0.0 (0.0-2.0)	NS (0.438)
	Gas GER	0.0 (0.0-2.0)	0.5 (00-2.0)	NS (0.313)
2nd PPH		**LLP:**	**RLP:**	
	TLESR	2.0 (1.0-5.0)	3.0 (1.0-7.0)	NS (0.250)
	All GER	2.5 (1.0-6.0)	4.0 (1.0-8.0)	NS (0.109)
	Liquid GER	0.0 (0.0-1.0)	3.5 (1.0-8.0)	0.004
	Mixed GER	0.0 (0.0-2.0)	0.0 (0.0-1.0)	NS (0.500)
	Gas GER	2.5 (0.0-6.0)	0.0 (0.0-1.0)	0.016
3rd PPH		**LLP:**	**RLP:**	
	TLESR	2.0 (0.0-8.0)	2.0 (0.0-6.0)	NS (0.922)
	All GER	2.0 (0.0-8.0)	1.5 (0.0-7.0)	NS (0.742)
	Liquid GER	0.0 (0.0-1.0)	1.0 (0.0-3.0)	0.016
	Mixed GER	0.0 (0.0-1.0)	0.0 (0.0-1.0)	NS (0.500)
	Gas GER	1.0 (0.0-7.0)	0.0 (0.0-4.0)	NS (0.164)

Table 2. Median (range) number of TLESRs and GER episodes. Comparison between the two protocols.
Differences expressed as p-value (Wilcoxon's matched pairs signed rank sum test). TLESR: transient lower esophageal sphincter relaxation; GER: gastroesophageal reflux; RLP: right lateral position; LLP: left lateral position; PPH: postprandial hour: NS: statistically not significantly different

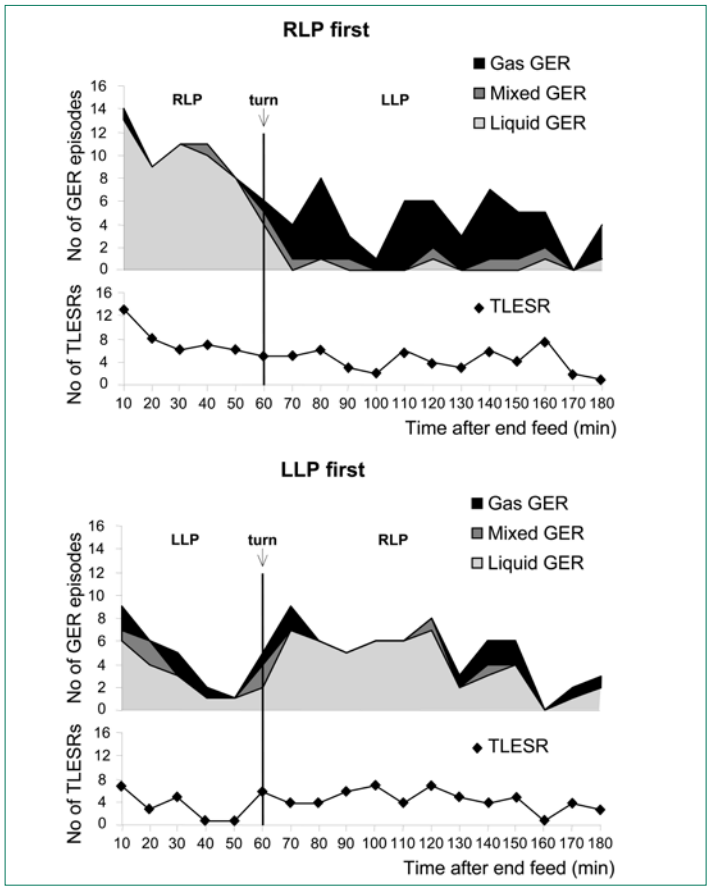

Figure 4: Total number of TLESRs and GER episodes (liquid, mixed and gas) over time. X-axis is comprised of 10 minute periods, where 10 stands for 0-10 minutes, 20 for 10-20 minutes etc. GER: gastro-esophageal reflux; LLP: left lateral position; RLP right lateral position.

RLP vs. LLP

More TLESRs and GER episodes were triggered while infants were in the RLP compared to the LLP [TLESRs: 9.5 (3.0-19.0) vs. 5.0 (3.0-17.0), respectively, p=0.020; GER episodes: 11.5 (6.0-24.0) vs. 7.0 (5.0-17.0), respectively, p=0.004]. Liquid GER episodes were more frequent in the RLP compared to LLP [9.5 (6.0-22.0) vs. 2.0 (0.0-5.0), respectively, p=0.002]. In contrast, the number of gas and mixed GER episodes was significantly lower in the RLP compared to LLP [gas: 0.0 (0.0-4.0) vs. 4.5 (1.0-10.0), respectively, p=0.012; mixed: 0.0 (0.0-2.0) vs. 1.0 (0.0-3.0) respectively, p=0.031]. The relative contribution of liquid, mixed and gas GER to the total number of GER episodes was therefore significantly different between the two positions (Figure 3).

RLP first protocol vs. LLP first protocol (Table 2)

The occurrence of TLESRs, as well as the number of GER episodes and the type of GER triggered over the study period differed between the two protocols (Figure 4). All data for the two protocols are given in Table 2.

First vs. second postprandial hour

During the RLP first protocol, the number of TLESRs and the number of liquid GER episodes were significantly higher, and gas GER episodes lower in the first hour (RLP) compared to the second (LLP) (Figure 4 & Table 3). During the LLP first protocol the number of liquid reflux episodes was significantly lower while the number of TLESRs and gas GER episodes was similar in the first hour (LLP) compared with the second (RLP) (Figure 4 and Table 3).

Gastric Emptying

Gastric half emptying time (GEt1/2) was significantly faster during the RLP first protocol when compared to the LLP first protocol (37.0 +/- 21.1 vs. 61.2 +/- 24.8, p=0.006 (Figure 5 and Table 4; online)).

RLP first protocol			
	1st PPH, RLP	2nd PPH, LLP	1st vs. 2nd PPH
TLESR	4.0 (2.0-8.0)	2.0 (1.0-5.0)	0.004
All GER	5.5 (2.0-15.0)	2.5 (1.0-6.0)	0.008
Liquid GER	5.5 (2.0-13.0)	0.0 (0.0-1.0)	0.002
Mixed GER	0.0 (0.0-2.0)	0.0 (0.0-2.0)	NS(1.000)
Gas GER	0.0 (0.0-2.0)	2.5 (0.0-6.0)	0.012
LLP first protocol			
	1st PPH, LLP	2nd PPH, RLP	1st vs. 2nd PPH
TLESR	2.5 (0.0-6.0)	3.0 (1.0-7.0)	NS(0.219)
All GER	2.5 (1.0-5.0)	4.0 (1.0-8.0)	NS(0.109)
Liquid GER	2.0 (0.0-4.0)	3.5 (1.0-8.0)	0.020
Mixed GER	0.0 (0.0-2.0)	0.0 (0.0-1.0)	NS(0.250)
Gas GER	0.5 (0.0-2.0)	0.0 (0.0-1.0)	NS(0.188)

Table 3: Median (range) number of TLESRs and GER episodes. Data given for 1st PPH and 2nd PPH.
Difference between 1st PPH and 2nd PPH expressed as p-value (Wilcoxon's matched pairs signed rank sum test). TLESR: transient lower esophageal sphincter relaxation; GER: gastroesophageal reflux; RLP: right lateral position; LLP: left lateral position,; PPH: postprandial hour.

	RLP first	LLP first	p-value
T1/2	37.0 ± 21.1	61.2 ± 24.8	0.006
Tlag	22.8 ± 6.7	27.0 ± 6.5	0.140
Tmax	58.4 ± 13.4	74.1 ± 17.1	0.012
GEC	3.8 ± 0.5	3.5 ± 0.3	0.056

Table 4. Gastric emptying parameters, RLP first protocol vs. LLP first protocol
Difference between RLP first and LLP first protocol expressed as p-value (paired t-test). RLP: right lateral position; LLP: left lateral position ; GEC: gastric emptying coefficient.

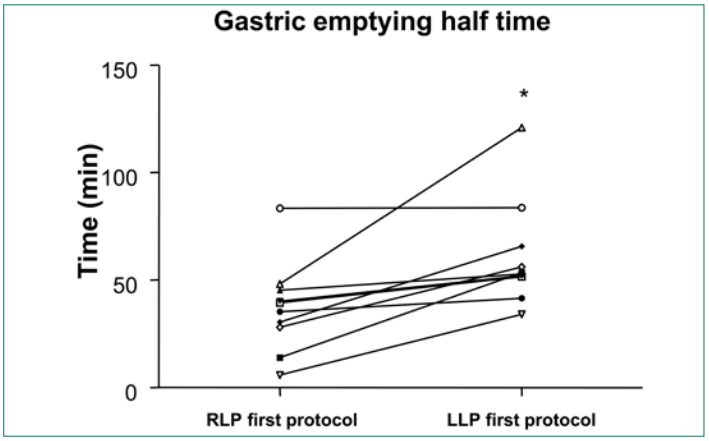

Figure 5: Gastric emptying half time, RLP first protocol vs. LLP first protocol. RLP right lateral position; LLP: left lateral position. p=0.006, paired t-test*

Discussion

This study shows that positioning healthy infants in the right lateral position (RLP) for the first postprandial hour (PPH) and in the left lateral position (LLP) afterwards virtually eliminates liquid GER in the late postprandial period when gastric contents are acidic. Overall, we found that more GER episodes were triggered in the RLP. This increase during RLP is in accordance with a recent study of healthy infants either in the RLP or the LLP, while we showed that more TLESRs are triggered in the RLP as compared with the LLP.[8]

In the present study, we have also shown that the proportion of liquid GER is much greater in the RLP as compared with the LLP (87% vs. 26%). This difference was not only present in the first PPH, but particularly became clear after the change to the left side position. Turning infants from the LLP to the RLP was associated with a rapid increase in occurrence of liquid GER episodes. On the other hand, turning the infants from the RLP to the LLP stopped liquid GER and caused triggering of gas GER. It is now clear that this change in reflux type relates to the anatomical configuration of the stomach which makes it more likely that liquid will pool at the level of the EGJ in the RLP.[9]

The current study has demonstrated that the change from left to right, as well as changing the type of GER triggered, also increased the total number of TLESRs and GER triggered. The opposite change from right to left had the opposite effect. In a dog model, Franzi *et al* showed that the threshold for distension-induced triggering of TLESRs is lowest in the region of the stomach just distal to the esophagogastric junction.[16] It could be argued that pooling of fluid at the EGJ region associated with a change to the RLP causes localized distension of the sub-cardiac region resulting in increased triggering of TLESRs. The opposite change to the LLP would result in pooling of fluid in the corpus and, although gas above the liquid would also distend the region just distal to the esophagogastric junction, the subsequent triggering of TLESRs and gas reflux would lead to greater depressurisation of the gastric lumen.[14]

Reduced capacity to vent gas and depressurise the stomach in the RLP could explain the rapid rise in TLESRs seen after the posture change to the right side. However, it should also be noted that GE rate also rapidly increased with the position change to the right. Although this increase in GE should assist depressurising the stomach, the triggering of TLESRs and GER is still increased. The opposite is the case with the position change to the LLP, during which the triggering of TLESRs and GER decreases, whilst at the same time GE slows.

Our previous studies in premature infants with and without GERD indicated that the triggering of acid reflux in association with TLESRs was significantly greater in infants with GER disease.[5,6] Therefore, control of acid GER in the late postprandial period may be more therapeutically relevant than reducing non-acid GER in the early postprandial period. In addition, it is a widely held view that delayed GE is a feature of GERD and that delayed GE also exacerbates reflux in GERD patients(17-19). The objective of any treatment for GERD should therefore be to reduce acid GER and accelerate GE. The current study indicates that this can be achieved in healthy infants by placing them in the RLP for one hour followed by a position change to the LLP.

In conclusion, our study showed that positioning a healthy infant on the RLP for the first PPH and turning it to the LLP afterwards, virtually eliminates all liquid GER in the late postprandial period, when acid GER occurs.

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Protein Energy Malnutrition in children: the proof of the pudding is in the eating

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Healthy humans eat to compensate for continuous loss of energy and nutrients due to ongoing mobilization and utilization of metabolic substrates from the tissues. Besides maintaining this status quo, children need to ingest extra amounts of energy and metabolic building blocks for growth (linear and body weight) and development (i.e. metabolic maturation of organs). In critical illness or after major surgery, energy and glucose are mobilized from fat tissue and glycogen storage pools. Proteins (predominantly from muscle) are degraded into amino acids that are funneled into the gluconeogenesis pathway, and serve as building blocks for new proteins (e.g. immunologic response, tissue repair). As apposed to fat and glucose, there is no resting storage pool of protein in the body. Since all proteins are functional (be it structural or soluble), loss of protein leads to loss of organ function (e.g. muscles, visceral organs) and thus of the organism as a whole. In critical illness, protein-energy malnutrition increases mortality and morbidity, number of ventilator days and length of stay (LOS) (1,2). Recent studies show that no less than 25% of patients on a Pediatric Intensive Care Unit (PICU) are malnourished at the time of admission, with risk of further deterioration of nutritional status during hospitalization (3,4). Inadequate feeding during the first few days of PICU admission accounts for almost 50% of cumulative caloric and protein deficits (5). Especially young infants and children after cardiac surgery, due to higher metabolic demands and fluid restriction, respectively, may be at risk for undernutrition (6). In a recent survey on our PICU, we found that caloric goals were met only on day 5 of admission, while the mean daily protein prescription remained 75% of age related targets during the entire admission period (maximum 10 days) (7).
Chronic illness is characterized by adaptive metabolic responses. It is well known that children with cystic fibrosis (CF) have less net protein anabolism than healthy individuals, resulting in stunted linear growth. However, this protein energy malnutrition has adverse effects on respiratory muscle strength, ventilatory drive, and immune defense mechanisms (8,9).
It is evident that in all different phases of illness there is a constant change of metabolic needs that have to be met by different amounts and composition of nutrition. In fact, clinical nutrition can be used to partly counteract disease-related catabolic effects by manipu-

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lation of breakdown and synthesis rates of tissues, via direct effect or by changes in serum levels of catabolic (e.g. cortisol) and anabolic hormones (e.g. insulin).
In children with acute disease (post cardiac surgery with extracorporeal circulation) and chronic disease (CF), we have observed the short-term effects of high (5 g·kg-1·d-1) versus normal dietary protein intake on whole-body protein synthesis and breakdown rates, and net protein balance (10,11). These effects on whole body protein metabolism were measured using stable isotopic infusion technique, which is explained elsewhere in detail (11). We also measured differences in serum concentrations of key anabolic and catabolic hormones in both disease states.

Critical illness

In a study in children, after cardiac surgery with extracorporeal circulation, we included 24 children, aged 3-48 months, with complex congenital heart defects (10). After allocation to either arm of the protocol (2 versus 5 g·kg-1·d-1 dietary protein intake), the study diets were started approximately 4 hours after surgery via a nasogastric tube (Fig.1). The diets consisted of a mixture of carbohydrate powder, fat emulsion, and whey protein powder, dissolved in water. Via the continuous drip-feeding the carbohydrate intake was held at a constant rate, in order to avoid great fluctuations of endogenous insulin secretion. The protein intake was varied according to protocol (2 and 5 g·kg-1·d-1, respectively), with the remaining non-protein calories supplied by fat.
After determination of background enrichment of natural isotopes, primed stable isotope infusion was started on the first postoperative day. On the second postoperative day, blood and breath samples were taken at regular intervals for determination of isotopic enrichment, and plasma concentrations of insulin, glucagon, and cortisol, and (nor)epinephrine. Isotopic kinetics of the normal protein (2 g·kg-1·d-1, NP) and high protein (5 g·kg-1·d-1, HP) groups showed no statistically significant differences in whole-body protein synthesis. However, in the HP group we observed a drastic reduction of endogenous protein breakdown with a consequent 11-fold more positive whole body protein balance, compared to the NP group. Moreover, patients in the HP group had a significantly higher serum concentration of the anabolic hormone insulin, and a lower concentration of the catabolic hormone cortisol. We concluded that in children after cardiac surgery, high dietary protein intake decreases short-term whole-body proteolysis, resulting in better net protein balance, potentially by means of increased insulin secretion, and decreased the plasma concentration of cortisol.

Chronic illness

In another study, we administered a 4-day liquid diet via a gastric continuous drip in eight chronically catabolic, stunted children with CF aged 7-12 years (11). The caloric intake of the study diet was 200% of the baseline measured energy expenditure, and contained a mixture of carbohydrate powder, fat emulsion, and protein powder. Via the continuous drip-feeding the carbohydrate intake was held at a constant rate, in order to avoid fluctuations of endogenous insulin secretion. The protein intake was varied according to protocol (1.5, 3 and 5 g·kg-1·d-1, respectively), with the remaining non-protein calories supplied by fat. On the fourth day whole body protein synthesis and breakdown were measured, by using stable isotope technique. The entire protocol was repeated twice in each child with a wash out period of six weeks, so that at the end of the study period, all children had been exposed to diets containing 1.5, 3 and 5 g·kg-1·d-1 protein.

In this study, we observed a progressive increase of whole-body protein synthesis of 30% with increasing dietary protein intake (1.5, 3 and 5 g·kg-1·d-1, respectively). On the other hand, whole-body protein breakdown differed not significantly between the diets, resulting in a higher net protein balance. There was a trend of higher serum concentrations of insulin and glucagons in the HP diet, compared to the NP diet.

Future research

In conclusion, we were able to improve whole-body protein balance in both study groups with supra-physiologic amounts of dietary protein intakes. However, in the post cardiac surgery patients, this was achieved by inhibiting proteolysis, whereas in the CF group protein synthesis was enhanced. In both study groups higher intakes of protein led to higher serum concentrations of insulin. Indeed, milk proteins have insulinotropic properties, of which the whey fraction contains the predominating secretagogue. In the cardiac surgery patients, there were also lower cortisol levels after the HP diet, compared to the NP diet.
These results show that protein balance can be improved in both acute and chronic illness by high protein diets. This is not the result of merely replenishing certain existing deficits with extra protein, but represents a remodeling action on the (hyper)metabolic stress response to illness in its different phases.
In future research, we would like to investigate whether this remodeling is a direct action of (a subset of) amino acids, or is induced via secretion of insulin.

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Verkort IB-tekst:

Samenstelling: CARNITENE bevat L-carnitine en is verkrijgbaar als: **CARNITENE** tabletten, 330 mg, **CARNITENE** drank 1 gram/ 10 ml (100 mg/ml) **CARNITENE** injectievloeistof, 1 gram / 5 ml (200 mg/ml). **Indicaties:** Primaire (systemische) carnitine deficiënties, **Eigenschappen: CARNITENE**, L-carnitine (γ-trimethylamino-B-hydroxybutyraat) is een lichaamseigen stof. L-carnitine wordt bij de mens hoofdzakelijk gevormd door endogene synthese uit lysine en methionine in de lever en de nier, maar kan ook verkregen worden uit de voeding. De L-isomeer, is biologisch actief en speelt een essentiële rol zowel in het lipide metabolisme als in het metabolisme van ketonlichamen en vertakte-keten aminozuren. L-carnitine is noodzakelijk voor het transport van lang-keten vetzuren over het binnenmembraan van de mitochondria naar de mitochondriale matrix, waar de B-oxidatie plaatsvindt met als resultaat productie van ATP. Bij een systemische carnitinedeficiëntie is er een tekort aan L-carnitine in het serum en in een of meerdere weefsels. De meest voorkomende symptomen van een systemische carnitine-deficiëntie zijn: 1. manifestatie begint in de eerste levensjaren, 2. acute episoden van encefalopathie (braken gevolgd door een progressief verlopende stupor, verwarring en coma) die geassocieerd wordt met leverfunctie stoornissen, zeer vaak geïnduceerd door een verminderde opname en/of fysieke inspanningen, 3. progressieve spierfunctie achteruitgang, 4. vetopstapeling in de spier- en andere weefsels (lever, nier enz.), 5. sterk verlaagde carnitinespiegels zowel in het bloed als in weefsel. Laboratorium onderzoek toont aan een hypoglykemie, een verhoging van CPK en leverenzymen in het serum, verhoogde ketose tijdens vasten, EMG (electromyogram) veranderingen.
De rationale om patiënten met een carnitinedeficiëntie te behandelen met L-carnitine ligt in het normaliseren van de weefselspiegels en/of deze in overeenstemming te brengen met de behoefte van het organisme op dat moment en de spierfunctie te herstellen. **Waarschuwingen voorzorgsmaatregelen:** Daar L-carnitine slechts in geringe mate gemetaboliseerd wordt, en als L-carnitine door de nier wordt uitgescheiden, wordt bij patiënten met een verminderde nierfunctie (GFR < 10 ml/min) aangeraden de medicatie te doen plaatsvinden op geleide van plasmaspiegels. Toediening van een hoge orale doses Levocarnitine gedurende lange perioden wordt niet aanbevolen in patiënten met een chronische nierinsufficiëntie, die gedialyseerd worden. Er vindt dan een cumulatie plaats van belangrijke metabolieten zoals trimethylamine (TMA) en trimethylamine-N-oxide (TMAO) omdat deze niet in voldoende mate door de nier geëlimineerd kunnen worden. Dit verschijnsel treedt niet in dezelfde mate op na intraveneuze toediening. Een cumulatie van TMA is nadelig omdat hiermee de stikstofhoudende afval producten die door dialyse verwijderd worden, verhoogd wordt. Bovendien worden de verhoogde TMA spiegels geassocieerd met neurofysiolosche effecten. De onvolledige eliminatie van TMA kan resulteren in de ontwikkeling van een vislucht geur. Indien overwogen wordt om deze patiënten levocarnitine toe te dienen wordt aangeraden dit intraveneus te doen**. Gebruik in de zwangerschap:** Over het gebruik van deze stof in de zwangerschap bij de mens bestaan onvoldoende gegevens om de mogelijke schadelijkheid te beoordelen. Er zijn tot dusver geen aanwijzingen verkregen voor schadelijkheid bij dierproeven. **Bijwerkingen:** Een lichte vorm van diarree bij sommige patiënten is na hoge orale toediening gerapporteerd. **Dosering:** De dosering wordt bepaald door de mate van carnitine deficiëntie. Indien mogelijk moet op geleide van carnitine bloed-/weefselspiegels behandeld worden.

Primaire (systemische) carnitine deficiëntie. Aanbevolen wordt de volgende dosering per os per dag:

Zuigelingen	100-150 mg/kg lichaamsgewicht
Kinderen tot 12 jaar	50-100 mg/kg lichaamsgewicht
Volwassen en kinderen boven de 12 jaar	20-40 mg/kg lichaamsgewicht

In de praktijk betekent dit dat de gemiddelde dosering per os per dag ligt voor:

Zuigelingen	1 gram
Kinderen tot 12 jaar	2 gram
Volwassen en kinderen boven de 12 jaar	2-4 gram (in twee a drie giften)

Indien er geen verbetering optreedt in de klinische en biochemische symptomen/spierzwakte, kan de dosering verhoogd worden tot 15 gram per dag, gedurende korte tijd. De **CARNITENE** injectievloeistof is bedoeld voor acute gevallen en wanneer toediening per os niet mogelijk is. De injectievloeistof dient langzaam (3 minuten) intraveneus toegediend te worden. Met de intraveneuze vorm kan met lagere doseringen (maximaal 30 mg/kg lichaamsgewicht per dag) worden volstaan, gezien de volledige beschikbaarheid van de stof na i.v. toediening ten opzichte van < 10% na orale toediening.

Onderverdeeld naar leeftijd betekent dit per dag:

Zuigelingen	maximaal 30 mg/kg lichaamsgewicht
Kinderen tot 12 jaar	maximaal 20 mg/kg lichaamsgewicht
Volwassen en kinderen boven de 12 jaar	maximaal 10 mg/kg lichaamsgewicht

Registratie: Ingeschreven in het register onder:

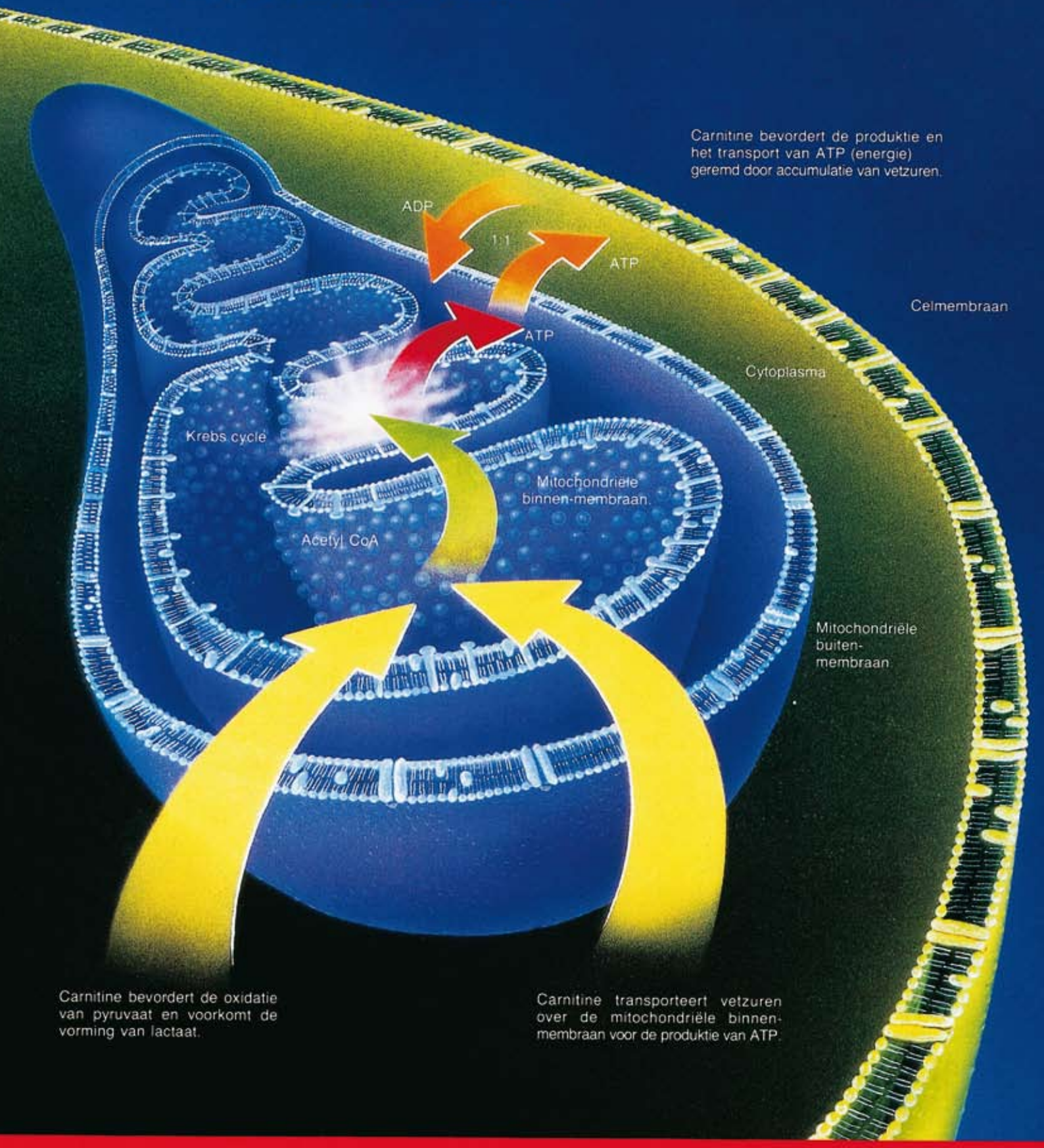
RVG 11192 **CARNITENE sigma tau** injectievloeistof	1 gram
RVG 11193 **CARNITENE sigma tau** drank	1 gram
RVG 11194 **CARNITENE sigma tau** tabletten	330 mg

Registratiehouder: Sigma Tau Ethifarma B.V., Postbus 10072, 9400 CB Assen. **Voor inlichtingen:** Sigma Tau Ethifarma B.V., Postbus 10072, 9400 CB Assen, telnr. 0592 333000.

sigma-tau
ETHIFARMA

DE CARNITINE ENERGIE CYCLUS

Carnitine is essentieel voor aerobe mitochondriële energie produktie en bevordert het transport van deze energie (ATP) in het cytoplasma, waar het de brandstof levert voor de cellulaire funkties.



CARNITENE sigma-tau®